Notes

A NEW ANTIBIOTIC, 13-HYDROXYGLUCOPIERICIDIN A

ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL CHARACTERISTICS

HIROHARU MORI,† SHINJI FUNAYAMA, YŌHEI SUDO, KANKI KOMIYAMA and SATOSHI ŌMURA*

The Kitasato Institute,
5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

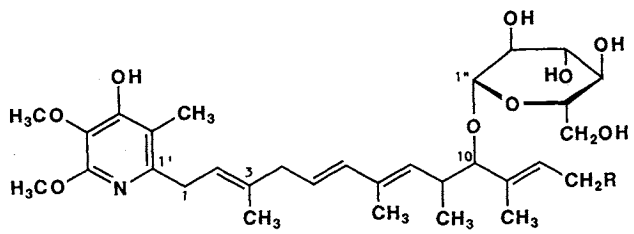
(Received for publication March 26, 1990)

In the course of our screening program for novel antitumor antibiotics, a new antibiotic, 13-hydroxyglucopiericidin A (**1**) was isolated from the fermentation broth of *Streptomyces* sp. OM-5689, together with previously isolated antibiotics, glucopiericidinols A_1 (**2**) and A_2 (**3**). The taxonomy and fermentation of the producing microorganism, *Streptomyces* sp. OM-5689 and isolation, structural elucidation and biological characteristics of **2** and **3** were reported in a preceding paper.[1] This paper deals with the isolation, structural elucidation and biological characteristics of 13-hydroxyglucopiericidin A (**1**).

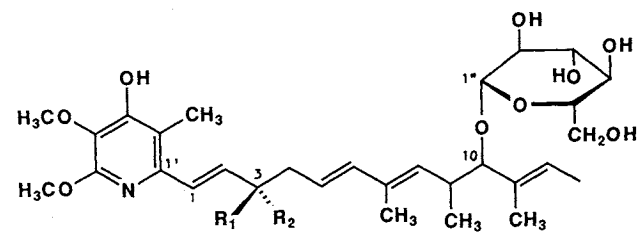
During fractionation of the cultured broth of *Streptomyces* sp. OM-5689, the existence of an additional glucopiericidin A analog was evident. The EtOAc extract (15.5 g) of the fermentation broth (70 liters) was chromatographed over silica gel, and fractions possessing cytocidal activity against B16 melanoma cells were collected. The active fractions were combined and chromatographed over Sephadex LH-20 and finally a new active component (170 mg) was purified by preparative TLC (DC-Fertigplatten Kieselgel 60 (Merck), $CHCl_3$-CH_3OH (9 : 1)).

The physico-chemical properties of this compound (Table 1) suggested that it was an analog of the glucopiericidins.[2] The molecular formula of this compound was the same as those for glucopiericidinols A_1 (**2**) and A_2 (**3**),[1] whereas UV spectral data were different from those of **2** and **3** and similar to those of glucopiericidins.[2]

The ^1H and ^{13}C NMR spectra of **1** were similar to those of glucopiericidin A (**4**)[2] and most of the signals were assigned straightforwardly (Table 2).



1 R=OH
4 R=H



2, **3** R_1, R_2=CH_3, OH

† Present address: Central Research Institute, Meiji Milk Products Co., Ltd., Sakae-cho, Higashimurayama-shi 189, Japan.

Spectral differences between **1** and **4** were found for signals in the C-11 ~ C-13 region (Fig. 1). In the ^{13}C NMR of **1** and **4**, a methyl signal at δ 13.2 assigned to C-13 of **4** was absent in the ^{13}C NMR of **1**, while a signal attributed to an oxygenated methylene was observed (δ 58.1 (t)). From these observations, the structure of the novel antibiotic was concluded to be 13-hydroxyglucopiericidin A (**1**).

13-Hydroxyglucopiericidin A (**1**) showed no antimicrobial activities at a concentration of 1,000 μg/ml against *Bacillus subtilis* KB27 (PCI 219), *Staphylococcus aureus* KB34 (FDA 209P), *Micrococcus luteus* KB40 (PCI 1001), *Mycobacterium smegmatis* KB42 (ATCC 607), *Escherichia coli* KB8 (NIHJ), *E. coli* KB176 (NIHJ JC-2), *Pseudomonas aeruginosa* KB105 (P3), *Xanthomonas oryzae* KB88, *Bacteroides fragilis* KB169, *Acholeplasma laidlawii* PG8 KB174, *Aspergillus niger* KF103 (ATCC 6275), *Mucor racemosus* KF 223 (IFO 4581), *Candida albicans* KF1 and *Saccharomyces sake* KF26, whereas **1** showed weak inhibitory activity against *Piricularia oryzae* KF180 at a concentration of 500 μg/ml (MIC). On the other hand, **1** showed strong cytocidal activity against various tumor cells *in vitro*. Inhibitory activity against *P. oryzae* KF180 and cytocidal activity against various tumor cells *in*

Table 1. Physico-chemical properties of 13-hydroxyglucopiericidin A (**1**).

Appearance	Yellow oil
TLC (silica gel)	
$CHCl_3$-MeOH (5:1)	0.65
BuOH-AcOH-H_2O (4:1:2)	0.70
$[\alpha]_D$	+20° (*c* 0.20, MeOH)
Molecular formula	$C_{31}H_{47}NO_{10}$
MW	593
UV λ_{max}^{MeOH} nm	239, 268
IR ν_{max} (smear) cm^{-1}	3400, 2930, 1585, 1470, 1410, 1125
Color reaction	
Positive:	Iodine, 50% $H_2SO_4+\Delta$, phosphomolybdic acid, DRAGENDORFF's reagent
Negative:	Ninhydrin reagent, $FeCl_3$

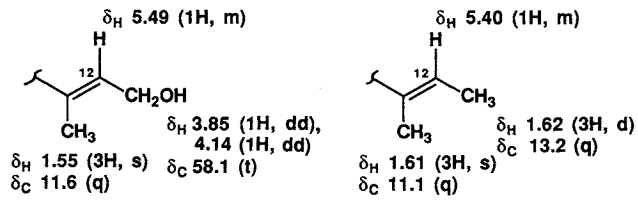
Table 2. NMR spectral data of 13-hydroxyglucopiericidin A (**1**)[a] and glucopiericidin A (**4**)[2] in $CDCl_3$.

Position	**1**		**4**
	δ_H (*J*/Hz)	δ_C	δ_C[2]
1	3.25 d (7) (2H)	34.5 t	34.5 t
2	5.25 t (7)	122.2 d	122.3 d
3		134.8 s	134.7 s
4	2.64 d (6) (2H)	43.0 t	43.0 t
5	5.46 dt (16, 7)	126.5 d	126.7 d
6	5.94 d (16)	135.5 d	135.7 d
7		134.3 s	134.4 s
8	5.15 d (10)	133.9 d	134.4 d
9	2.64 m	35.0 d	35.3 d
10	3.44 d (9)	92.9 d	94.2 d
11		138.6 s	135.4 s
12	5.49 m	127.5 d	123.3 d
13	3.85 dd (13, 5), 4.14 dd (13, 9)	58.1 t	13.2 q[c]
14 (11-CH_3)	1.55 s	11.6 q	11.1 q
15 (9-CH_3)	0.68 d (7)	16.9 q	17.0 q
16 (7-CH_3)	1.61 s	16.6 q	16.6 q
17 (3-CH_3)	1.64 s	13.1 q	13.0 q[c]
1′		150.9 s	150.8 s
2′		112.3 s	112.3 s
3′		154.2 s	154.2 s
4′		127.9 s	128.0 s
5′		153.7 s	153.6 s
6′ (2′-CH_3)	1.96 s	10.5 q	10.5 q
7′ (4′-OCH_3)	3.71 s	60.6 q	60.5 q
8′ (5′-OCH_3)	3.82 s	53.1 q	53.1 q
1″	4.09 d (7)	103.5 d	103.7 d
2″	3.15 m	74.0 d[b]	74.4 d[d]
3″	3.34 t (8)	76.2 d	75.5 d
4″	3.15 m	70.6 d	70.8 d
5″	3.15 m	76.3 d[b]	76.4 d[d]
6″	3.48 dd (12, 6), 3.69 d (12)	62.4 t	62.5 t

[a] Assignments were based on comparison with the literature.[2]

[b~d] Assignments may be interchanged.

Fig. 1. Partial structures of 13-hydroxyglucopiericidin A (**1**) and glucopiericidin A (**4**).



1 **4**

Table 3. Biological activities of 13-hydroxyglucopiericidin A (**1**), glucopiericidinols A_1 (**2**) and A_2 (**3**) and glucopiericidin A (**4**).

	1	**2**	**3**	**4**
Cytocidal activities (IC_{50}: μg/ml)				
HeLa S_3 human cervices carcinoma	0.76	0.62	0.98	0.11
B16 murine melanoma	0.21	0.32	0.67	0.0074
H-69 human lung carcinoma	0.066	0.47	0.83	0.019
P388 murine leukemia	2.5	0.58	1.6	0.36
P388/ADM murine leukemia	0.78	4.3	4.2	0.25
Antimicrobial activity (MIC: μg/ml)				
Piricularia oryzae	500	125	31	31

vitro of **1** and related compounds are summarized in Table 3.

It is interesting that 13-hydroxyglucopiericidin A (**1**) showed lower inhibitory activity against *P. oryzae* KF180, whereas it showed strong cytocidal activities against various tumor cells *in vitro*. It is especially noteworthy that **1** showed stronger activity (IC_{50}, 0.78 μg/ml) against P388/ADM cells than P388 (IC_{50}, 2.5 μg/ml).

We are now investigating the *in vivo* antitumor activities and other biological properties of this compound and the results will be published elsewhere.

Acknowledgment

This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan, and by funds from the Japan Keirin Association.

References

1) FUNAYAMA, S.; M. ISHIBASHI, Y. ANRAKU, M. MIYAUCHI, H. MORI, K. KOMIYAMA & S. ŌMURA: Novel cytocidal antibiotics, glucopiericidinols A_1 and A_2. Taxonomy, fermentation, isolation, structure elucidation and biological characteristics. J. Antibiotics 42: 1734~1740, 1989

2) MATSUMOTO, M.; K. MOGI, K. NAGAOKA, S. ISHIZEKI, R. KAWAHARA & T. NAKASHIMA: New piericidin glucosides, glucopiericidins A and B. J. Antibiotics 40: 149~156, 1987